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Molecular Modelling-Based Investigations of a Mutant Protein in Patients with Hearing Loss

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1. Introduction

The inner ear is a most complicated and highly organized anatomical region that possesses an elaborate system for hearing. It requires a unique structure for discriminating between the physical vibrations caused by various sounds and specific cellular mechanisms to convert the physical sounds received into chemical signals that must be transmitted to the brain correctly. For normal hearing, the highly optimized environment is controlled by the concerted function of an enormous number of expressed proteins. If proteins comprising the elements of hearing are damaged, it is possible that the normal hearing function might be impaired.

So, how do sound waves travel through the ear canal and integrate in the inner ear? Vibrations that come into contact with the tympanic membrane travel through the air-filled middle ear cavity via auditory ossicles: namely, the malleus, incus and stapes. These ossicles convert lower-pressure eardrum sound vibrations into higher-pressure sound vibrations at a smaller membrane called the oval window. This window is a membrane-covered opening that leads from the middle ear to the vestibule of the inner ear or cochlea (Figure 1a, b, c). The sound waves are transduced into nerve impulses, which are perceived by the temporal lobe of the brain. A core component of the cochlea is the Organ of Corti—the sensory organ of hearing—which is distributed along the partition that separates fluid chambers in the coiled, tapered tube of the cochlea (Figure 1d). Sound waves passing through the scala timpani in the cochlea vibrate the tectorial membrane and stimulate stereocilia, which are located on the periplasmic region of hair cells (Figure 2). A shearing movement between the tectorial membrane and the basilar membrane deflects the stereocilia, affecting tension on the tip-link filaments, which then open and close non-

specific ion channels [1]. When the tension increases, the flow of ions across the membrane into the hair cell also rises. This is the first step of the mechanoelectrical transduction system and causes receptor depolarization, which subsequently excites the cochlear nerve afferents that are located at the base of the hair cell. In the cochlea, many specific genes such as ion channels, mechanical proteins and signal transduction-related molecules, among others, are expressed and function in hearing [2]. In the case that more than one of these proteins is mutated, hearing function might be impaired or lost. This is one of the main causes of congenital hearing loss, which can be inherited.

It is widely known that deafness is a frequently inherited sensory disorder: one in every 500 new-borns has bilateral sensorineural hearing loss (SNHL) and 70% of these cases are congenital [3, 4]. Hereditary hearing loss is classified as either syndromic or nonsyndromic [5]. It is assumed that hearing-associated genes amount to over 400 [6]; to date, 76 genes involved in syndromic and nonsyndromic hearing loss have been identified [7]. The genetic causes of nonsyndromic hearing loss are autosomal dominant (27 identified genes), autosomal recessive (40 genes), X-chromosome-linked (three genes) and mitochondrial (six genes) [7].

The expression patterns of genes in the inner ear can be visualized on the Hereditary Hearing Loss Homepage [7]. In terms of genes associated with deafness, many different types of mutation have been identified, including missense, nonsense, splicing, regulatory, deletions, insertions, indels and duplications. So far, missense/nonsense mutations have been shown to account for 55.4% of all mutation types [2]. Identification of phenotype-genotype correlations is crucial for determining the aetiology of congenital hearing loss and has implications for prognostic and therapeutic outcomes. For missense mutations in the coding regions of a gene, it is assumed that the mechanism of hearing loss is based on functional disorders of the gene product. Thus, to understand congenital hearing loss it is essential to study extensively the functions of proteins expressed in the inner ear.

Hearing levels and patterns are represented by an audiogram, which is a graph showing the results of pure-tone hearing tests (Figure 3). Hearing loss can be categorized in terms of which part of the auditory system is damaged. There are three basic types of hearing loss: conductive hearing loss, SNHL and mixed hearing loss. In most cases, hearing loss is SNHL and nonsyndromic [8]. It is clear that some correlations between hearing loss and specific genes are robust, such as the low-frequency audioprofile associated with WFS1-related hearing loss [9] and the mid-frequency audioprofile associated with TECTA-related hearing loss [10]. High-frequency hearing loss, by contrast, can be the consequence of mutations in a large number of different genes such as KCNQ4, DFNA5, COCH and POU4F3 [9]. As part of an approach to determining how mutations of these proteins cause dysfunction in the inner ear, analysis of their three-dimensional structures is indispensable.

Analysis of protein structures (i.e., by X-ray crystallography, NMR, cryo-EM, etc.) is very effective in elucidating the mechanisms of hearing loss. However, the reported protein structures that are related to hearing loss account for only 16 of the 97,789 structures deposited in the Protein Data Bank (PDB) [11]. Most hearing-related proteins are adhe-

sion molecules (e.g., Gap junction, CDH23), ion transporters (e.g., KCNQ4, SLC26A4) or proteins involved in vesicular transport such as SNARE proteins (e.g., Otoferlin) [12, 13], for which it is difficult to elucidate the three-dimensional structure owing to technical difficulties such as protein expression, purification and crystallization, among others. Thus, the protein structures of hearing-related proteins are not well elucidated.

However, it is possible to generate computer-predicted structures of disease-related proteins if appropriate template structures are available in the PDB and also to elucidate the mechanism of protein function impairment by comparing wild-type and mutant structures. This alternative approach has an advantage in that the association of ligands such as sugar chains with a protein is extremely difficult to examine by crystallization, due to the structural mobility of the sugar chain, whereas it can be easily investigated by docking simulations. Thus, *in silico* modelling can help explain the functional impairment caused by mutation in terms of protein structure.

In this chapter, we describe a protein, namely a voltage-gated potassium channel, which is implicated in hearing loss. We discuss how mutants of this protein from patients cause hearing impairment from the point of view of molecular structure. Several types of software are freely available for these kinds of study and are useful for pharmacologists, molecular biologists and physicians. In addition, information on the three-dimensional structure is essential for discovering seed compounds in the field of drug design. Elucidation of structural data and the mechanisms of functional impairment due to mutations, coupled with computer-aided drug design, can lead to clinical benefits.

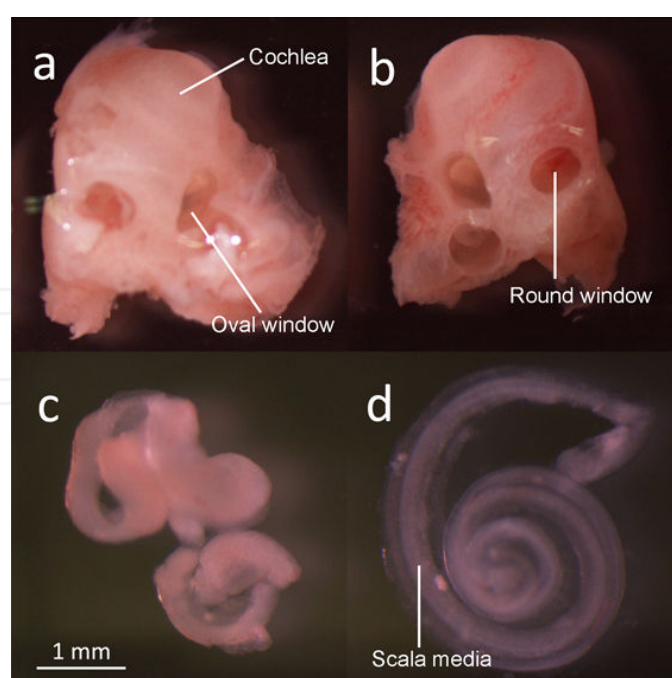


Figure 1. Structures of the rat's inner ear. (a, b): Whole-mount structure of the left (a) and right (b) inner ear with bone tissue dissected from rat. (c, d): The vestibular organ (c) and cochlea (d) extracted from bone tissues.

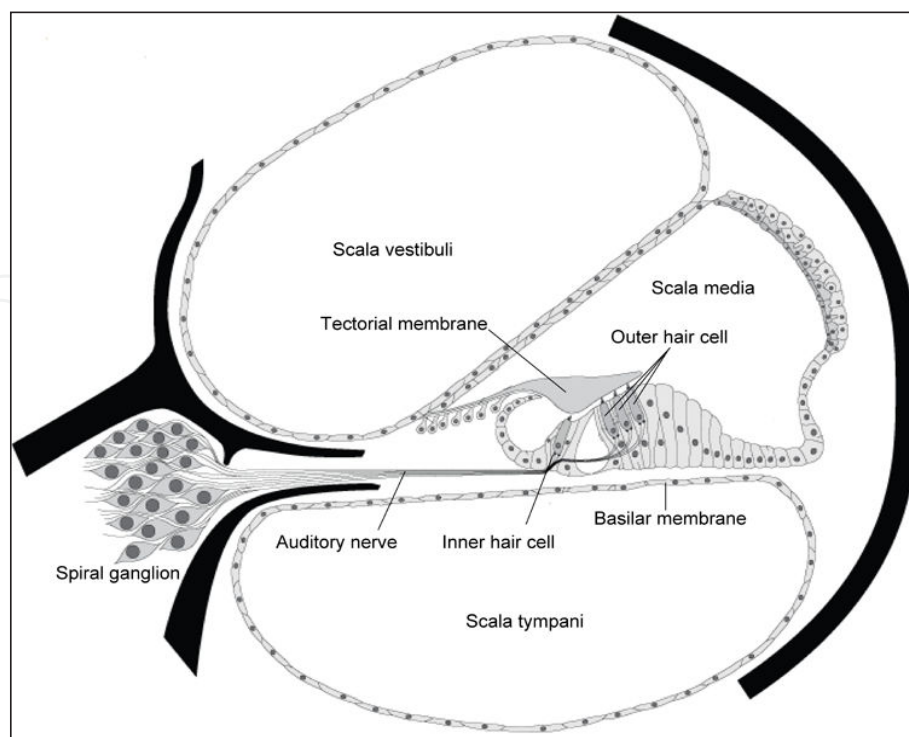


Figure 2. Schematic diagram of the coronal section of the cochlear duct. The majority of hearing loss-related genes are expressed in the cochlea. Hereditary Hearing Loss Homepage [7]

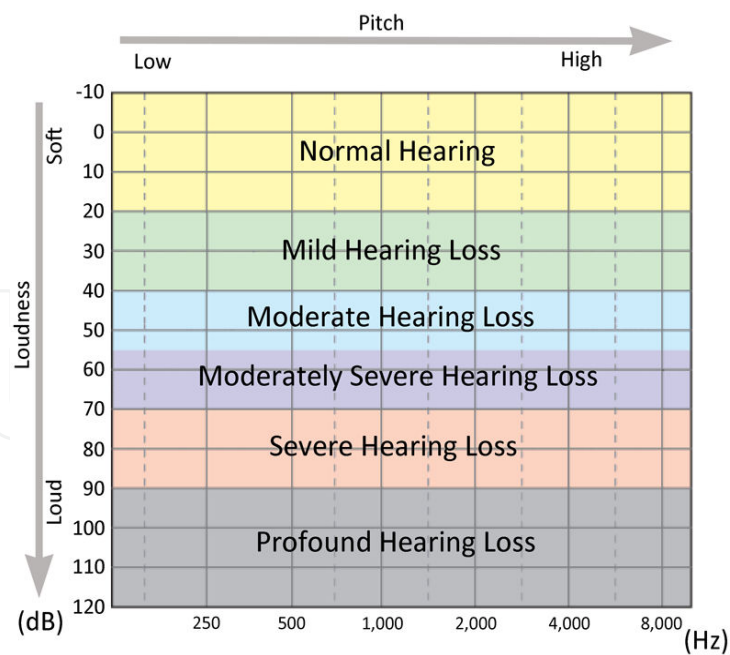


Figure 3. Hearing levels expressed on an audiogram. Frequency is expressed in terms of the number of cycles per second, or "Hertz". The range of most of the sounds in speech is 250Hz to 8000Hz. Loudness or "level of sound" is measured in units called decibels (dBs). The audiogram shows hearing loss across a range of frequencies; both the hearing level and the threshold of hearing level are indicated.

2. The voltage-gated potassium channel KCNQ4

KCNQ4, which encodes the voltage-gated potassium channel KQT-like subfamily member 4, has been identified as a causative gene in hearing loss [14]. The gene encodes a protein of 695 amino acid residues in its longest isoform and contains six transmembrane α -helices, (S1–S6), a pore helix (PH), a pore-loop (P-loop), a short N-terminal region and a long C-terminal region (Figure 4). As in other KQT-like channels, the ion-selective channel formed by KCNQ4 comprises a tetramer of identical subunits in which the highly conserved P-loop of each subunit combines to form the pore structure [15]. The pore region of the KCNQ4 channel consists of contiguous structures of S5, PH, P-loop and S6 elements. Functional and structural analyses of Kv channels have demonstrated that the PH and the P-loop are responsible for the selective K^+ penetration, whereas S4 regulates the open-closed state of the channel as a voltage sensor [16–18].

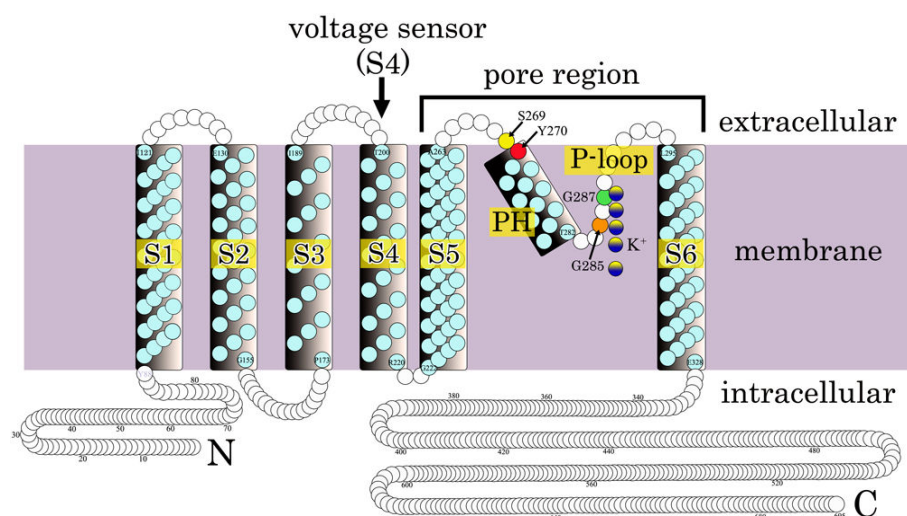


Figure 4. Schematic representation of the six transmembrane helices (S1–S6) and the K^+ selective channel pore region (S5, PH, P-loop and S6) of KCNQ4. Yellow, red, orange and green circles indicate the p.Ser260del, p.Y270H, p.G285S and p.G287R mutations, respectively. Dark blue spheres indicate potassium ions. This figure was made with some modification using the figure in our previous study [23].

KCNQ4 is expressed predominantly in the basolateral membrane of outer hair cells, a type of auditory sensory cell in the cochlea (Figure 5) and plays an important role in the proper electrophysiological function of these cells [19]. KCNQ4 is also expressed in spiral ganglion (SG) cells, although its function in SG cells is unknown. A mouse model expressing a dominant-negative form of KCNQ4 demonstrates hearing loss with slowly progressive degeneration of the outer hair cells owing to chronic depolarization caused by loss of the major K^+ efflux pathway [20]. The clinical features—namely, congenital, progressive high-frequency sensorineural hearing loss (Figure 6) without substantial loss of speech recognition during the first decade of life—exhibit a very strong hereditary tendency for patients with mutated KCNQ4 proteins [21, 22]. Since patients with KCNQ4 mutations show progressive hearing loss, the

development of a drug to improve the function of the KCNQ4 channel might attenuate the symptoms.

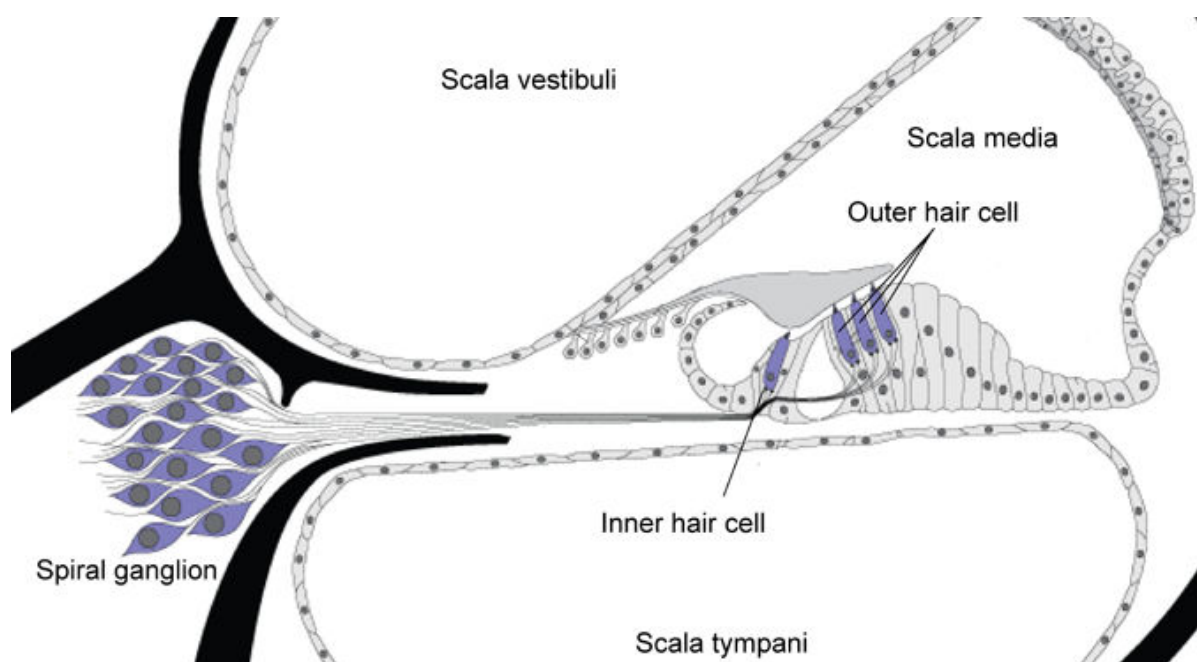


Figure 5. Expression pattern of the KCNQ4 channel in the cochlea. Protein expression is indicated in purple.

We identified two disease-associated mutations of KCNQ4: in one, a tyrosine residue (Tyr270) is replaced with histidine (His); and in the other, there is a deletion, c.806_808delCCT, leading to a p.Ser260del located between S5 and the pore helix (PH) in the gene product of *KCNQ4* [23, 24]. We then generated a computational structural model of the KCNQ4 channel by referring to the crystal structure of the Shaker family K^+ channel, Kv1.2 [23, 24]. In the following sections, we speculate about the molecular mechanism underlying hearing loss according to the basic quantum chemistry of a KCNQ4 channel formed with the Tyr270His (p.Y270H) and p.Ser260del (p.S269del) mutations and we discuss how two more severe mutations-p.Gly285Ser (p.G285S) [14] and p.Gly287Arg (p.G287R) [25]-might cause severe to profound hearing loss from a structural point of view.

3. Materials and methods

3.1. Genetic analysis

Initially, KCNQ4 was selected as a candidate gene involved in hearing loss on the basis of clinical features [26]. Prior to this study, the patient was confirmed to have neither *GJB2* mutations, the most common causative gene of hereditary hearing loss, nor the mitochondrial m.1555A > G and m.3243A > G mutations. Genomic DNA was extracted from blood samples

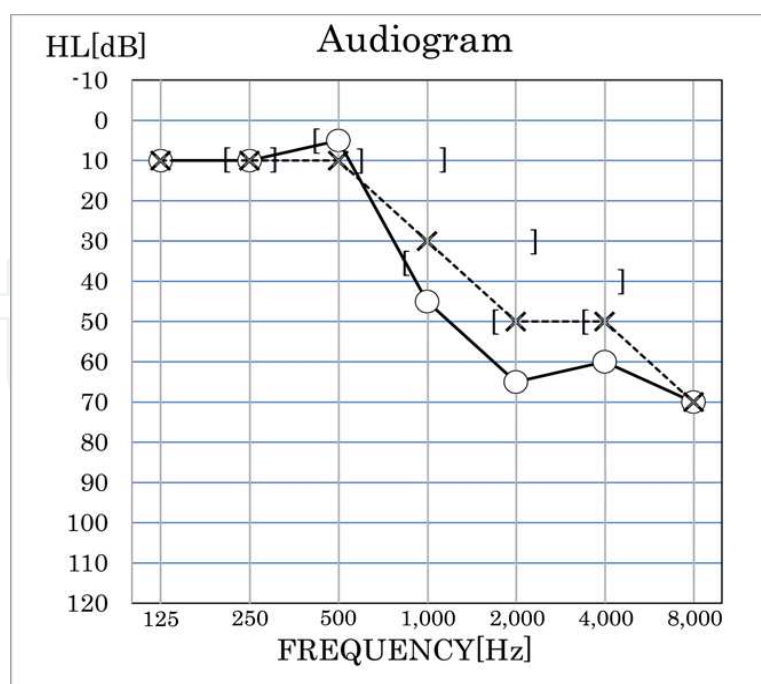


Figure 6. An audiogram from a patient with the KCNQ4 Tyr270His mutation [23]. Open circles indicate the hearing levels of air conduction for the right ear. Crosses indicate the hearing levels of air conduction for the left ear. “[” and “]” indicate the hearing levels of bone conduction in the right and left ear, respectively. In conductive hearing loss, the bone conduction hearing thresholds are normal but there is a loss of hearing for air conduction sounds. This means that the cochlea is normal, but there is a blockage to sound in the middle or outer ear. This audiogram shows that the patient has sensorineural hearing loss (SNHL) because the hearing pattern of air conduction is close to that of the bone conduction.

via the Gentra Puregene Blood Kit (QIAGEN, Hamburg, Germany). PCR primers specific for KCNQ4 were designed in our laboratory [23].

3.2. Molecular modelling of KCNQ4

A series of molecular modelling was conducted by the following procedures.

1. **PDBsum:** To find structural relatives of KCNQ4 for molecular modelling, we utilized PDBsum [27] and Gapped BLAST [28] in an attempt to search for the protein that is most homologous in amino acid sequence to the full length of KCNQ4 and that has a macromolecular structure deposited in the Protein Data Bank.
2. **Swiss-Model:** Among the channels with available three-dimensional structures, the sequence of Kv1.2 was most similar to that of KCNQ4 (27.5% identity for the transmembrane sequence Ser32 to Thr417). The transmembrane domains (S1–S6) including the voltage sensor and the pore region of KCNQ4 (Tyr80 to Gln329) and its Tyr270His and p.Ser260del mutants were modelled using the fully automated protein structure modelling server Swiss-Model [29–31] and the crystal structure of Kv1.2 (PDB ID: 3LUT, chain B) as a template [17]. The structural model of KCNQ4 was calculated by automatic modelling mode with default conditions.

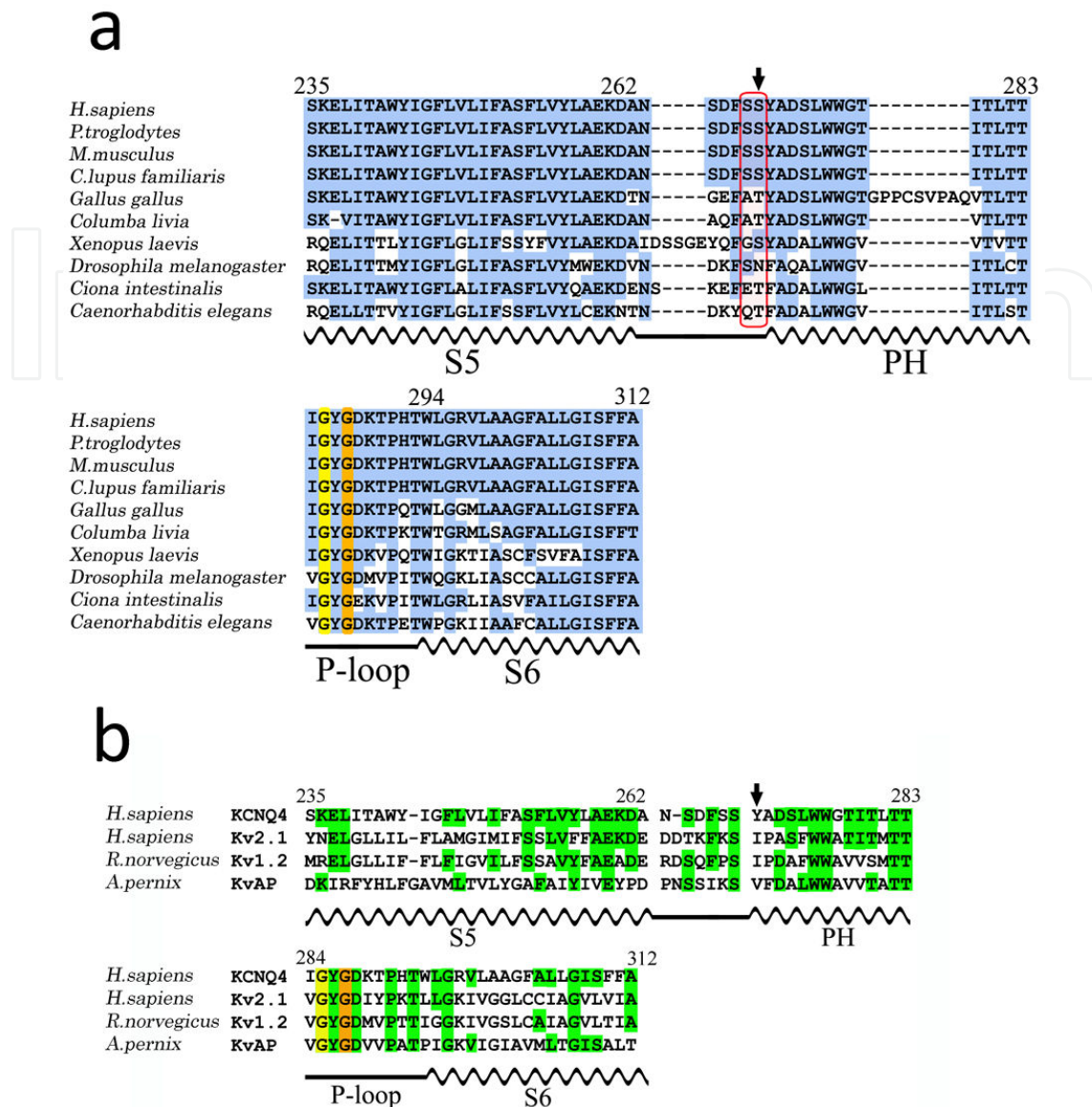


Figure 7. Sequence alignment of KCNQ4 proteins. (a) Sequence alignment of the orthologous KCNQ4 pore region. Positions highlighted in light blue indicate that the amino acid is identical to that in human KCNQ4. The position of Ser269 and Tyr270 is boxed in red and Tyr270 is indicated by an arrow. The positions of Gly285 and Gly287 are highlighted in yellow and orange, respectively. The positions of S5, PH, S6 (wavy lines) and the P-loop (straight line) are indicated below the sequences. (b) Alignment of the KCNQ4 pore-region sequence with those of Kv channels deposited in the PDB. Positions highlighted in green indicate that the amino acid is identical to that in human KCNQ4. The sequence of human KCNQ4 is indicated at the top of both alignments. This figure was made with some modification using the figure in our previous study [23].

3. **Verify_3D Structure Evaluation Server:** The quality of the structural model was evaluated by Verify_3D [32-34] and was found to be trustworthy [23].
4. **UCSF Chimera:** The structural models of KCNQ4 and the Tyr270His, p.Ser260del, p.G285S and p.G287R mutants were fitted into the corresponding transmembrane domains of the Kv1.2 structure and UCSF Chimera [35] was used to visualize the putative structure by α -carbon frame or by ribbon model with electric surface potentials.

4. Results and discussion

To date, 21 missense mutations, a splice-site mutation and four small deletion mutations in KCNQ4 have been reported to be associated with hearing loss [36]. Eleven of the missense mutations are located in the pore region; thus, these mutations are considered to be pathogenic [14, 21, 23, 24, 37, 38]. As mentioned above, KCNQ4 contains six transmembrane α -helices, (S1–S6), a pore helix (PH), a pore-loop (P-loop), a short N-terminal region and a long C-terminal region (Figure 4). The pore regions of Kv channels are formed by contiguous S5, PH, P-loop and S6 regions. Functional and structural characterizations of Kv channels have demonstrated that the PH and P-loop are responsible for selective K⁺ transport, whereas S4, acting as a voltage sensor, regulates the open–closed state of the channel [17, 18]. Tyr270 and Ser269 are predicted to be the N-terminal residues of the PH.

Among the mammalian KCNQ4 sequences that are available, the pore-region sequences are identical and those of non-mammals (e.g., birds, fruit flies, tunicates and nematodes) are very similar to the mammalian sequences (Figure 7a). In addition, the PH and P-loop sequences of non-KCNQ4 Kv channels are somewhat conserved (e.g., Kv2.1 from *Homo sapiens* is identical in 14/24 amino acid residues, Kv1.2 from *Rattus norvegicus* in 11/24 residues and KvAP, a prokaryotic Kv channel from *Aeropyrum pernix*, in 13/24 residues). In addition, the positions of Gly285 and Gly287 on the P-loop are fully conserved across mammalian and non-mammalian KCNQ4 sequences. These commonly mutated residues are thought to be important for proper functioning of the channel (Figure 7a).

Tyr270, at the N-terminus of PH, is located at the same position as the corresponding Kv1.2 residue (Ile361) (Figure 7b). Gly285 and Gly287 are also fully conserved in Kv channels. We therefore assessed the electrostatic characteristics of structural models containing mutations of these residues to determine whether these residues might be responsible for the patient's hearing loss.

4.1. p.Y270H mutation

The ribbon model of wild-type KCNQ4 with the electrostatic surface potential superimposed suggests that the side chain of Tyr270 (coloured white in Figure 8a) should be electrostatically neutral. For Tyr270His, by contrast, the side chain of His270 (coloured blue in Figure 8b) is predicted to retain at least a partial positive charge, which reflects the standard pK_a value (6.5) for histidine.

Moreover, His270 is surrounded by the negatively charged residues Asp272, Asp266, Asp262 and Glu260, as well as the polar residues Ser269, Ser268 and Ser265, which are capable of hydrogen bonding (all of these residues are within 10 Å of His270, Figure 8c). In haemoglobin (PDB ID: 2hhb), the side-chain pK_a of His97, which is surrounded by two negatively-charged asparagine residues within five Å distance, is shifted to an abnormally high value (pK_a =7.8) [39]. For these reasons, the side chain of His270 may also have a pK value that is greater than the standard value; therefore, it is predicted that more than half of the histidine side chains at position 270 carry a positive charge at near physiological pH (Figure 8b).

Alteration of the electrostatic surface potential of a single helical residue in the pore region might affect the structural stability of the channel as a consequence of a change in the helix dipole moment. A substantial dipole moment with positive and negative unit charges at, respectively, the N- and C-terminus of an α -helix is often found (Hol, 1985). A comparison of three Kv channel structures suggests that the dipoles of the transmembrane helices orient the helices and are responsible, at least in part, for the structure of the pore [15, 40, 41]. The electrically neutral Tyr270, which, as noted above, is positioned at the N-terminus of the PH, is located within 9 Å of Ala263 at the C-terminus of S5 (Figure 8a, b). The presence of a positively-charged histidine residue adjacent to, or at the N-terminal of, an α -helix can destabilize the helix dipole [42]. Replacement of Tyr270 with histidine may therefore increase the dipole moment of the PH.

Alternatively, or in conjunction with the destabilization effect, His270 may impede K⁺ transport. The electrostatic repulsion between positively-charged His270 and K⁺ would be stronger than that between electrically-neutral Tyr270 and K⁺. In the p.Y270H model, the distance between His270 and the centre pore of the channel is ~20.5 Å (Figure 8d, e). This distance is small enough to affect the electrostatic interaction between two charged molecules in a non-polar environment (e.g., the interior of a membrane protein) because the electrostatic force is strong and affects charges separated by 500–1800 Å [43–45]. In addition, the dielectric constants in the interior of a protein and in a lipid membrane are assumed to be five and two, respectively, whereas that of bulk water is ~80 at room temperature [46], a value that can be elicited by basic Coulomb's law (see Formula). The electrostatic potential energy for two charges in protein or lipid membrane interiors is therefore between 16 and 40 times greater than that in water. Consequently, the long-range electrostatic repulsive force between positively charged His270 and a K⁺ ion might possibly impede passage of K⁺ through the channel [47], whereas the force on K⁺ would be smaller in the extracellular region.

Several mutations in the PH of KCNQ4 (e.g., Leu274His and Trp276Ser) have been correlated with hearing loss [21]. Interestingly, ectopic expression of both the Trp276Ser mutant and wild-type KCNQ4 in a cultured cell line caused a reduction in the channel current, whereas expression of the mutant alone caused impaired trafficking of the protein to the cellular membrane [48]. The similar clinical symptoms and locations of the mutations within KCNQ4 support the proposal that p.Y270H is a pathogenic mutation associated with progressive SNHL. Further physiological and three-dimensional structural characterization of the p.Y270H KCNQ4 protein may identify which of our working hypotheses—namely, structural distortion of the channel caused by a change in the dipole moment of the PH or electrostatic impediment of K⁺ transport (or both)—causes hearing loss in the patient and may provide insight into how to reverse hearing loss caused by KCNQ4 mutations.

Formula: (1) The electrostatic repulsive interactions in the pore region can be explained by classic Coulomb's law. Tyr270 in KCNQ4 is neutrally charged, whereas His270 in the Tyr270His mutation is predicted to be positively charged (at physiological pH 7.4). Thus, extra electrostatic repulsion force (q) is generated between His270 and K⁺, which is more intense than that between Tyr270 and K⁺.

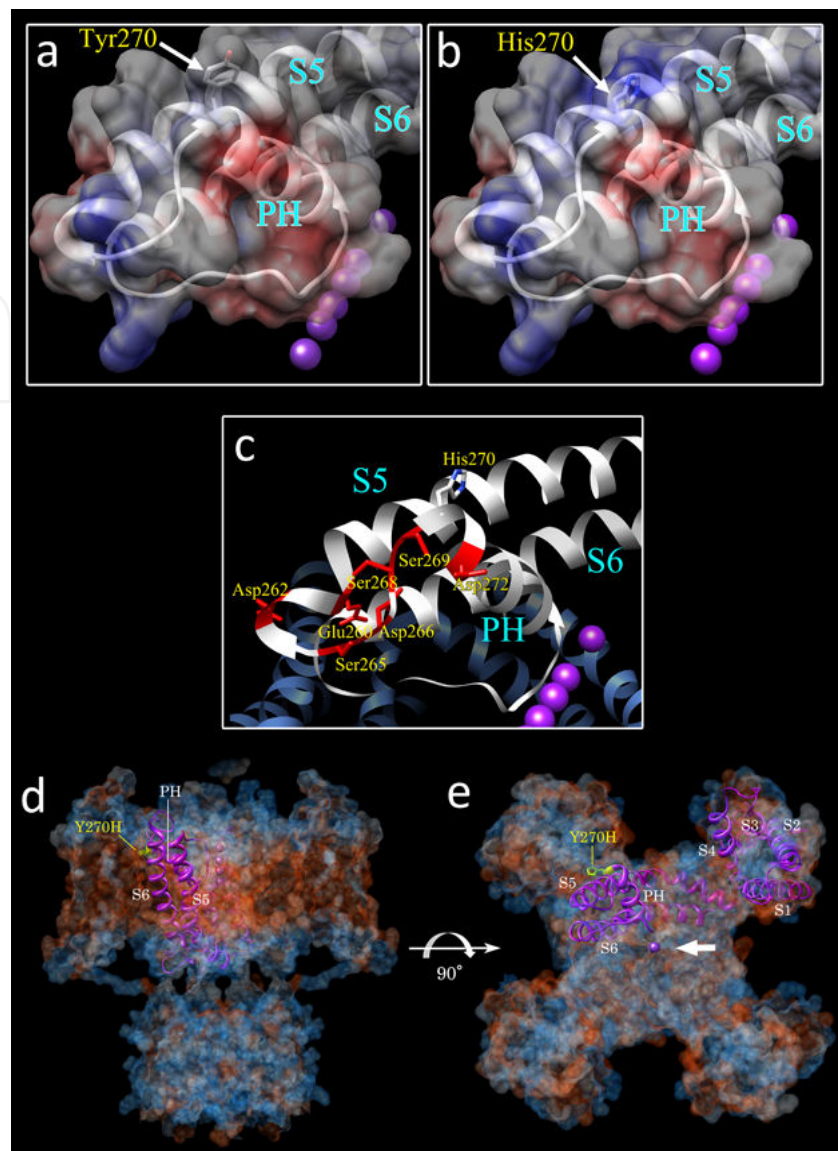


Figure 8. Structural models of KCNQ4 and the Tyr270His mutant. (a, b) Part of the wild-type KCNQ4 model (a) and the Tyr270His model (b) overlaid with their corresponding electrostatic surface potentials. Arrows indicate the side chains of Tyr270 (a) and His270 (b). Blue, red and white indicate positively-charged, negatively-charged and neutral atoms, respectively. (c) Ribbon model of the Tyr270His pore region. Residues surrounding His270 that are negatively-charged or that can form a hydrogen bond are shown in red. (d, e) Superimposition of the Tyr270His ribbon model onto a horizontal view of the plasma membrane (d) and an extracellular view (e) with the four rotational axes of the Kv1.2 crystal structure shown as a hydrophobicity surface. Hydrophobic and hydrophilic residues are indicated in red and blue, respectively, in the hydrophobicity surface representations. His270 is shown in yellow. K⁺ in the central pore is shown as a purple sphere in a–e and indicated by an arrow in e. This figure was made with some modification using the figure in our previous study [23].

$$\begin{aligned}
 q_Y q_{K^+} &\approx 0 \\
 q_H q_{K^+} &> 0 \\
 q_H q_{K^+} &> q_Y q_{K^+}
 \end{aligned}
 \tag{1}$$

(2) The electrostatic potential energy (E) between His270 and K^+ is affected by the dielectric constant (ϵ).

$$E = \sum_{i < j} \frac{q_i q_j}{4\pi\epsilon r_{ij}} \quad (2)$$

(3) Assuming that the K^+ ion is within the pore region of the K^+ channel, the ϵ value is 5, whereas in water, the ϵ value is 80. Therefore, the electric repulsive force would be much more intense between His270 and K^+ within the pore region of the K^+ channel than in the extracellular space.

$$E_{pore} = \sum_{i < j} \frac{q_H q_{K^+}}{4\pi 5 r_{ij}} \gg E_{water} = \sum_{i < j} \frac{q_H q_{K^+}}{4\pi 80 r_{ij}} \quad (3)$$

(4) The Van der Waals potential energy (V) between His270 and K^+ can be ignored because it is extremely weak as compared with the electrostatic force. This energy decays exponentially with the internuclear distance (r). A and B represent the repulsive and attractive constant, respectively.

$$V(r) = \frac{A}{r^{12}} - \frac{B}{r^6} \quad (4)$$

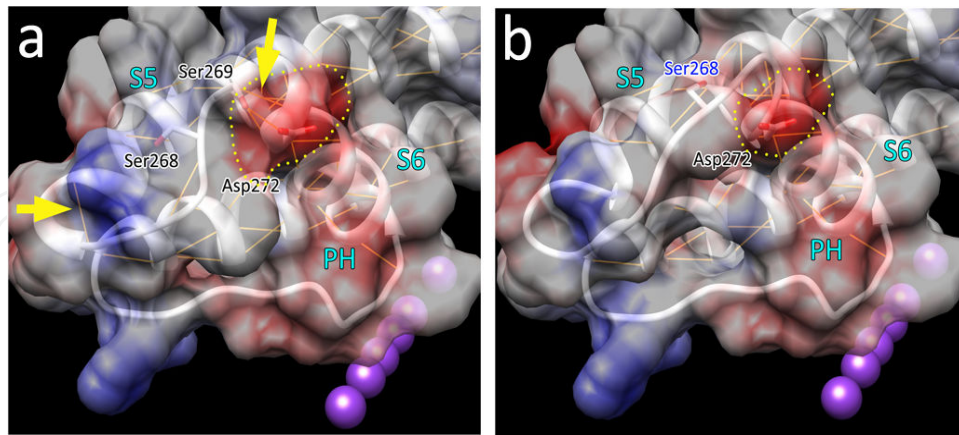


Figure 9. Partial structural model of KCNQ4 and the p.Ser269del mutation. (a, b) Ribbon models of the wild-type KCNQ4 subunit (a) and the KCNQ4 subunit with the p.Ser269del mutation (b) overlaid with their corresponding electrostatic surface potential. Red or blue areas indicate negatively- or positively-charged residues, respectively; yellow dots indicate the negatively-charged surface potential on the N-terminal region of the PH; pale yellow lines indicate hydrogen bonds and yellow arrows indicate hydrogen bonds within S5 and PH. This figure was made with some modification using the figure in our previous study [24].

4.2. p.Ser269del mutation

The ribbon model of the wild-type KCNQ4 subunit overlaid with the corresponding electrostatic surface potential demonstrates that the surface of the N-terminal region of the PH is negatively charged owing to the negatively-charged side chains of Ser269 and Asp272 (Figure 9a). The model of KCNQ4 with the p.Ser269del mutation demonstrates a reduction in the negatively-charged surface area in this region (Figure 9b). In case of the p.Y270H mutation, reducing the electrostatic surface potential in this area is predicted to impede K⁺ transport owing to the long-range electrostatic force between the PH and K⁺ (Formula).

In addition, hydrogen bonds at the C-terminus of S5 and the N-terminus of the PH of wild-type KCNQ4 (Figure 9a, yellow arrows) are absent in Ser269del KCNQ4 (Figure 9b). Loss of the hydrogen bonds around the N-terminus of the PH results in shortening of the PH and has been attributed to the destabilization of α -helix formation, resulting in a change in the helix dipole moment [39]. A change in dipole moment in this case might also destabilize the pore region structure, which is also likely to impede K⁺ transport. Overall, the molecular impairment is likely to be a mild dominant-negative effect, resulting from the relatively small influence of the p.Ser269del mutation on the normal KCNQ4 channel subunit.

4.3. p.G285S and p.G287R mutations

Of particular importance in the P-loop are the three amino acids GYG, which are highly conserved in the Kv channel family (Figure 7b). The structure derived from molecular modelling reveals that the GYG residues (Gly285 and Gly287) bind directly to K⁺ and this electric static level is an integral part in the normal function of K⁺ penetration. In the p.G285S mutant structure, the electrostatic value of glycine is very similar to that of serine; however, the side chain of serine is polarized (Figure 10a, b: yellow arrow). This side chain protrusion of serine changes the normal structure of the central route of K⁺, providing decisive evidence of the impediment of K⁺ transport. In support of this, the current measured by electrophysiological examination in *Xenopus* oocytes was markedly reduced by the dominant-negative effect of p.G285S as compared with wild type [14].

The pK_a value for Arg (12.48) is positively charged in normal periplasmic environment and generates a strong repulsive force between Arg287 and K⁺ (Figure 10c). In addition, the side chain of Arg287 protrudes into the K⁺ position, resulting in complete blockage of the passage of K⁺. Thus, the effect of the p.G287R mutation is much stronger than that of the p.G287S mutation, from the structural analysis data. However, the hearing levels and patterns of patients with the p.G285S and p.G287R mutations are very similar [14, 25].

At the genetic level, KCNQ4 channels with missense mutations are predicted to act via a dominant-negative mechanism to induce progressive, predominantly high-frequency hearing loss [14]. In families with deletions that lead to frameshifts and stop codons, however, the phenotype is characterized by better low-frequency deterioration, but more rapid high-frequency deterioration [22, 49]. In addition, it is thought that truncating mutations such as p.Gln71SerfsX138 and p.Gln71fs on heterozygous alleles are probably not synthesized from these alleles and functional impairment is considered to be due to haploinsufficiency [24, 37,

50]. Missense mutations are usually translated into protein, whereas nonsense and large truncating mutations are not. For a heteromeric missense mutation of KCNQ4, incomplete tetramers might be generated; however, the functional structure of KCNQ4 is a tetramer of protein subunits. If one mutated subunit is integrated in the tetramer, the function of the K⁺ transportation will be lost, even though three subunits are still normal. Thus, a dominant-negative effect occurs. In the case of haploinsufficiency, a normal tetramer of KCNQ4 will be expressed, although the allelic expression is 50% of the normal value. These possibilities indicate that differences in hearing patterns and levels might occur between missense and nonsense (or large deletion) mutations.

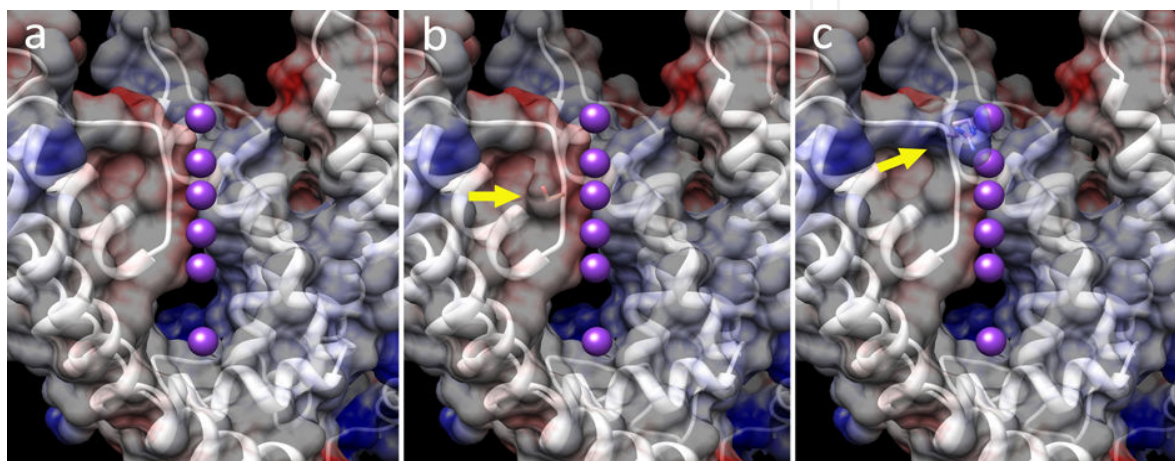


Figure 10. Structural model of the centre pore region of KCNQ4. The wild-type KCNQ4 (a), the p.G285S mutation (b) and the p.G287R mutation (c) models are overlaid with their corresponding electrostatic surface potentials. The side chains of Ser285 (b) and Arg287 (c) are indicated by arrows. The periplasmic region is at the top.

5. Conclusion

From our structural analysis, KCNQ4 is a transporter that has been refined during protein evolution. To develop molecular drugs targeted at, for example, the pore region of KCNQ4, it is necessary to take a special approach other than the normal screening of seed compounds as candidate inhibitors of a pathogenesis factor. Considering the molecular interpretation described in this chapter, it seems that there are two approaches to the development of drugs that might act on mutations in the P-loop in a target. The first is a drug that acts on the P-loop directly. However, it is presumed that the development of drugs directly targeting the P-loop would be somewhat difficult because the P-loop is hypersusceptible and there is no space in the centre pore for K⁺ passage. The second and ideal approach is to screen for compounds that act on mutations on the P-loop at long range, taking into consideration our p.Y270H mutation model. It is hoped that such a drug that can control the penetration of ions from a remote position might be developed in future studies. In this chapter, we have described a case of structural impairment caused by KCNQ4 mutation. Elucidation of the impairment mechanism

underlying missense mutations and the strategy of drug design will be applicable to the development of therapies for many genetic illnesses.

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